UNIVERSITY OF HAWAI'I AT MĀNOA

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To: Ms. Nancy Rumrill
U.S. Environmental Protection Agency
Ground Water Office (WTR-9)
75 Hawthorne Street
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From: Meghan Dailer, Junior Researcher
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Re: Public Comments on Lahaina, HI WWRF UIC Permit Number HI50710003

Thank you for the opportunity to comment on the permit request.

Based on summary information presented below, we ask that the UIC permit for the Lahaina Wastewater Treatment Plant be revised to decrease the total allowable daily nutrient load, including but not limited to: Total Nitrogen, Total Phosphorous, Manganese, and Iron for the reason that these components are macronutrients and micronutrients apparently stimulating algal growth in coastal regions around the Lahaina Wastewater Treatment Plant.

Background: Algal blooms and decline of the reef at Kahekili Maui.

Nuisance algal blooms of the red alga *Hypnea musciformis* and the green alga *Ulva fasciata* are problematic in shallow coastal waters around urbanized regions of Maui. Currently the South Maui blooms span from North Kihei (near the NOAA Sanctuary office buildings) to Charley Young Beach and then from Keawekapu through Wailea. Central Maui blooms occur in Maalaea to the south and from Kahului to Paia in the north. Research on these algae blooms thus far have included laboratory and field experiments as well as sampling bloom and non-bloom areas for nutrients in water column and pore water of sediments , algal tissue carbon, nitrogen, phosphorus and stable isotopes of nitrogen (^{15}N:^{14}N, expressed as $\delta^{15}N$). We collected these data in two ways - as 'on-shore / off-shore gradients' and spatially along the coastline from La Perouse to Waipuilani.

The North Kaanapali Beach (also called Kahekili) area is in the review process to become a Fisheries Management Replenishment Area (FMRA) through the State of Hawaii, Department of Land and Natural Resources, Division of Aquatic Resources to increase herbivore pressure on algal growth. This area was chosen for the FMRA because of the significant decline in coral cover and accompanying abundance of algae over the past decade. In the summers, when large north swells no longer persist and the south swells are fewer and farther between, the shallow fore reef (approximately 5 to 30 feet offshore) becomes dominated with algae blooms, primarily comprised

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of *Ulva fasciata,* but historically *Hypnea musciformis* and *Cladophora sericea* as well. This area also frequently has bubbles flowing from the benthos and warmer-than-ambient-water fresh water seeps. The seeps are consistently present and are surrounded by rocks and coral rubble with black precipitates. The black precipitate is currently being analyzed, but is likely iron oxide which could arise from anoxic conditions in the groundwater.

Examining $\delta^{15}N$ values of tissue nitrogen in intertidal macroalgae on Maui to identify locations and potential sources of nutrient enrichment

Overview: Because nitrogen is often present in low concentrations in tropical marine waters but is an essential macronutrient for plant growth, macroalgae can acquire nitrogen from many sources such as land based fertilizers and sewage effluent when present in coastal waters. These sources of additional nitrogen entering the ocean are often difficult to detect with many water quality assessment tools (ambient nutrient and salinity measurements) because coastal currents, wave action and general mixing events dilute potentially elevated nutrient levels quickly. However, natural stable isotopes of nitrogen (^{15}N:^{14}N, expressed as $\delta^{15}N$) enable researchers to detect anthropogenic nitrogen loading because nitrogen sources can differ in their $\delta^{15}N$ signatures (Umezawa et al. 2002, Lin et al. 2007, Gartner et al. 2002). For example, $\delta^{15}N$ signatures of sewage derived wastewater range from 11to 25‰, and can be as high as 38‰ (Savage and Elmgren 2004). The $\delta^{15}N$ values of macroalgae growing directly in front of sewage outfalls are often highly enriched with values ranging from 9 to 15‰ (Lin et al. 2007, Gartner et al. 2002, and Costanzo et al. 2001), well above background values for ocean waters. Because macroalgae continuously acquire nitrogen from their surrounding waters, the $\delta^{15}N$ values of algal tissues are an integration of all nitrogen sources available to them. Because these sources are integrated over time by the algal physiology, the $\delta^{15}N$ values of macroalgae are more useful in detecting anthropogenic sources of enrichment than monitoring dissolved inorganic nitrogen levels in the water (Umezawa et al. 2002, Gartner et al. 2002).

Summary of Methods: In the summer of 2007, a survey of intertidal macroalgal $\delta^{15}N$ values from all accessible coastlines on Maui was conducted to locate areas and potential sources of anthropogenic enrichment. A total of 130 sites (over 600 samples) were sampled around the island and at each site attached macroalgae samples were collected in triplicate from the intertidal zone. Samples were prepared for laboratory analysis and sent to the University of Hawaii Manoa, Isotope Biogeochemical Laboratory for analysis. Stable isotope composition (^{15}N:^{14}N) was determined using the following instrumentation: the Carla Erba NC2500 Elemental Analyzer via Finnigan MAT ConFloII system and the Finnigan MAT DeltaS.

Summary of findings: Average macroalgal $\delta^{15}N$ values generally reflect the areas' exposure to anthropogenic impact (Figure1). The $\delta^{15}N$ value of samples from Olowalu, La Perouse and Haleakala National Park, areas of low anthropogenic impact were low (blue circles). The $\delta^{15}N$ values to the north of the Lahaina Wastewater Treatment Plant (LWTP) decreased from 6 to 5 ‰ moving north. In marked contrast to those low values north of LWTP, the $\delta^{15}N$ values of samples collected from the north end of Kahekili Beach Park, slightly south of the LWTP decreased from 43.26 ± 0.24 ‰ to 34.66 ± 0.13 ‰ as plant collections were made to the south away from the LWTP. The $\delta^{15}N$ values of samples to the south of the treatment plant markedly exceed those reported for other sewage affected areas elsewhere in the literature. The highest $\delta^{15}N$ values reported thus far are approximately 38 ‰ for secondarily-treated sewage and 25.7‰ ± 3.8 ‰ for macroalgae in an estuary due to anthropogenic nitrogen loading from the Scheldt River (Savage and Elmgren 2004 and Riera et al. 2000, respectively).

The use of $\delta^{15}N$ values in algal tissues to detect, monitor, and map anthropogenic sources of nitrogen is a new and growing field of research which may limit our ability to find comparably enriched values to those found at Kahekili. However, many studies have linked enriched signals in macroalgae to the presence of sewage effluent with values two to three times lower than Kahekili. The most recent research by Lin et al. (2007) in Taiwan, Gartner et al. (2002) in Western Australia and Costanzo et al. (2001) in Eastern Australia reports highly enriched macroalgae values ranging from 9.30 to 14.9 ‰ directly in front of sewage outfalls (Note: the volume and concentrations of nutrients at that site remain unknown, limiting the ability to compare absolute values of $\delta^{15}N$ from tissues samples).

Investigating the physiological response of *Hypnea musciformis* to additions of reagent grade Nitrogen and Phosphorous

A nutrient enrichment experiment was conducted to explore the effects of Nitrogen (N) and Phosphorus (P) independently and in combination on the growth and photosynthetic properties of *Hypnea musciformis*. In this experiment, separate samples (n=9, per treatment) of *H. musciformis* were grown in an outdoor aquarium system in individual aerated 1.0L beakers under one of the following nutrient treatments for seven days: (1) No Addition, (2) Mid Phosphorus (MP: 0.5μMPO_4), (3) Mid Nitrogen (MN: 40.0μMNH_4), (4) MNMP (40.0μMNH_4 and 0.5μMPO_4)(5) High Phosphorus (HP: 1.0μMPO_4), (6) High Nitrogen (HN: 80.0μMNH_4), and (7) HNHP (80.0μMNH_4, 1.0μMPO_4). On Days 0 and 7 samples were weighed (wet weight) and assessed for (1) photosynthetic status, Relative Maximum Electron Transport Rate ($RETR_{MAX}$) and (2) Photosynthetic Saturation Irradiance (E_K) with a Pulse Amplitude Modulated (PAM) fluorometer (Diving PAM, Waltz). The water in each 1.0L beaker was changed every day of the experiment to maintain the desired nutrient concentrations.

The response of *Hypnea musciformis* on Day 7 to the abovementioned treatments is visually displayed in Figure 2 where the coloration of plants given any combination of N is clearly distinct from those lacking N addition. In fact this change in color from dark purple to tan is nearly complete by Day 4. The fact that *H. musciformis* maintains phycobilin pigmentation (dark purple color) only when given nitrogen allows for the potential to use this plant as an indicator species of elevated nitrogen levels. Surprisingly, there were no significant differences of growth between the No Addition treatment and any other treatment. The highest sustained $RETR_{MAX}$ and E_K values were observed in the MNMP and HNHP treatments on Day 7. The E_K values of samples in the MNMP and HNHP treatments were significantly higher than all other treatments on Day 7.

This study documented how rapidly *H. musciformis* responded to elevated combined N and P conditions with increases in pigmentation leading to increased photosynthetic capabilities. These findings confirmed the expected requirement of both N and P to sustain increases in photosynthesis. The surprising lack of significant differences among growth rates between the No Addition and other treatments initiated many additional nutrient enrichment experiments with N, P, and Iron (Fe) in every combination as well as different light treatments. None of those experiments produced the growth rates observed in the field supporting nuisance algae blooms.

Investigating the response of *Hypnea musciformis*, *Ulva fasciata*, *Acanthophora spicifera*, and *Dictyota acutiloba* to secondarily-treated sewage effluent

Overview: The abovementioned Maui coastline survey of intertidal macroalgae successfully detected elevated $\delta^{15}N$ values of samples that were likely influenced by sewage effluent percolating into the near shore marine environment in certain areas. Yet we were unable to reproduce observed growth rates of plants in the field while conducting laboratory nutrient enrichments. For this reason, we pursued the response of bloom species and non-bloom species to secondarily treated sewage effluent. *Hypnea musciformis* (invasive, bloom forming), *Ulva fasciata* (native, bloom forming), *Acanthophora spicifera* (invasive, bloom forming), and *Dictyota acutiloba* (native, common but not bloom forming).

All species were separately subjected to a series dilution experiment to determine their response to sewage effluent in terms of growth, photosynthetic status, $\delta^{15}N$ values, and nutrient uptake rates. In addition, we planned to determine if the tissue composition (C:N:P) of the plants are representative of the surrounding available nutrient concentrations, and what micro-nutrients (Zinc, Iron, Molybdenum, Manganese, Magnesium, and Copper) limit photosynthesis and growth in these species.

Summary of Methods: The following processes were repeated for each species. Samples were subjected to the following 7 treatments (n = 6 per treatment): (1) No Addition, (2) 25ml, (3) 50ml, (4) 75ml, (5) 100ml, (6) 150ml, and (7) 200ml of sewage effluent. Treatments for each sample were refreshed every day with (1) water from Olowalu (area of low anthropogenic impact), (2) the corresponding sewage effluent addition (obtained from the Lahaina Wastewater Treatment Plant) and (3) the appropriate addition of natural sea salt to return the salinity to oceanic levels (32 ‰, confirmed with a Mettler Toledo Seven Multi meter (calibrated with Mettler Toledo conductivity standards).

Trials lasted for 9 days with the following sampling and measuring design. On Days 0 and 9 samples were first measured with Pulse Amplitude Modulated (PAM) fluorometry for photosynthetic parameters (1) Relative Electron Transport ($RETR_{MAX}$), (2) Photosynthetic Saturation Irradiance (E_K), and (3) Photosynthetic Efficiency (Alpha) then weighed. Samples were prepared in triplicate per treatment for $\delta^{15}N$ and tissue nutrient analysis on Days 0 and Day 9. To determine that the sewage effluent additions remained consistent during the study samples for water chemistry analysis were collected in triplicate per treatment on Days 0 and 8 for analysis of the following macro and micro nutrients: Total Organic Carbon (TOC), Total Nitrogen (TN), Total Phosphorous (TP), Nitrate (NO3), Iron (Fe), Molybdenum (Mo), Manganese (Mn), and Copper (Cu). Nutrient uptake rates were determined in triplicate per treatment over a 24 hour time period by collecting water samples on Days 8 and 9 from the same samples at the same time.

Summary of Results: Samples of *Hypnea musciformis*, *Acanthophora spicifera* and *Ulva fasciata* visibly change with distinct increases in pigment in only six days (Figures 3 -6). Highly significant differences in the Relative Growth Rates (RGR) (Figure 7A) between Days 0 and 9 were found between the No Addition and all treatments above 25 and 50 ml for *H. musciformis* and *U. fasciata*, respectively, while no significant difference was found among any treatment for *A. spicifera* or *D. acutiloba*. In addition, the RGRs of *H. musciformis* and *U. fasciata* from the 50 ml through the 200 ml additions were significantly higher than those of *A. spicifera* and *D. acutiloba*. This shows that in terms of growth, *H. musciformis* and *U. fasciata* similarly respond to excess nutrients more positively and faster than *A. spicifera* and *D. acutiloba*.

Relative Maximum Electron Transport Rates ($RETR_{MAX}$) (Figure 7B) of *U. fasciata* and *H. musciformis in* the 200 ml addition were significantly accelerated from their No Addition treatment, while *A. spicifera* $RETR_{MAX}$ values in treatments 75 to 200 ml were significantly higher than the No Addition treatment. No significant differences were found between the No Addition and any treatment for *D. acutiloba*. *U. fasciata* had significantly higher $RETR_{MAX}$ values than all other species in treatments of elevated nutrients (25, 50, and 200 ml). *H. musciformis* and *D. acutiloba* $RETR_{MAX}$ values were not significantly different in any treatment. These results indicate that (1) *U. fasciata* increases photosynthetic performance with fewer nutrients than what is required for both *H. musciformis* and *A. spicifera*, (2) from the No Addition to the 150 ml treatment, $RETR_{MAX}$ values of *H. musciformis* and *D. acutiloba* were similarly unaffected by increased nutrients, and (3) $RETR_{MAX}$ values of *A. spicifera* increased similarly to those of *U. fasciata* in treatments of 75 ml and above.

The Photosynthetic Saturation Irradiance (E_K) (Figure 7C) in the 200 ml addition was significantly higher than the No Addition treatment for all species except *D. acutiloba*. In addition, all species had significantly higher E_K values than *D. acutiloba* in the 200 ml treatment. The highest E_K values were found in *U. fasciata* in the 200 ml treatment. In the 75 ml treatment, *A. spicifera* E_K values were significantly higher than those of *D. acutiloba* and the No Addition treatment. This shows that all species, except for *D. acutiloba*, positively respond to excess nutrients in terms of building photosynthetic capacity and *U. fasciata* is the most responsive.

No difference between treatments was found in the Alpha values (Figure 7D) of *D. acutiloba*, *H. musciformis*, or *A. spicifera U. fasciata*, however, had significantly lower values between the No Addition and all other treatments. *D. acutiloba* had significantly higher Alpha values than *H. musciformis* regardless of treatment. This shows that (1) *U. fasciata* is more sensitive to decreased nutrient conditions in terms of photosynthetic efficiency than all other species and (2) *D. acutiloba*, the native, high light environment (reef flat) macroalgae, has higher photosynthetic efficiency than the bloom forming algae.

The water chemistry results from the *Hypnea musciformis* component of this study (Figures 8 and 9) found no significant difference was found between Treatment or Treatment and Day for Total Organic Carbon (TOC, Fig. 8A), Total Phosphorous (TP, Fig. 8B), Copper (Cu, Fig. 9B), and Molybdenum (Mo, Fig. 9D). Significant differences were found between Treatment (same day comparisons only) and Days 8 and Day 9 (same treatment comparisons only) in Total Nitrogen (TN, Fig. 8C), Nitrate (NO_3, Fig. 8D) and Iron (Fe, Fig. 9A). The Manganese (Mn, Fig. 9C) concentrations significantly increased with Treatment on Days 0 and 8. The TN concentrations on Days 0 and 8 in the No Addition, 25 ml, and 50ml treatments were significantly lower than the additions of 75 ml and above. In addition, the TN significantly decreased in treatments of 75ml and above from Day 8 to Day 9. NO_3 concentrations on Days 0 and 8 significantly increased with treatment and on Day 9 were significantly decreased from those on Day 8 in all treatments from 50 ml and above. Fe concentrations were significantly increased from the No Addition treatment in the 150 and 200 ml treatments. A significant decrease in Fe from Day 8 to 9 was found in the 200 ml treatment. These results show that *H. musciformis* is capable of utilizing substantial amounts of N, P, Fe, and Mo over a 24 hour time period. These results also suggest that micronutrients such as Mn play a previously overlooked key role in the growth and photosynthetic properties of the invasive bloom forming macroalga, *H. musciformis*.

Overall Summary

Nuisance algal blooms of the red alga *Hypnea musciformis* and the green alga *Ulva fasciata* are problematic in shallow coastal waters around urbanized regions of Maui. The Kahekili area is an area of problematic algal growth and substantial reef decline. Kahekili has the highest macroalgal $\delta^{15}N$ values on Maui, which strongly indicates the presence of sewage effluent in the near shore marine environment. Sewage effluent contains elevated levels of many nutrients, some of which are important for algal growth and photosynthetic needs. From laboratory studies with reagent grade nutrient enrichment, we see that Nitrogen and Phosphorous play important roles in the photosynthetic needs of *Hypnea musciformis*, but are unable to promote excessive growth by themselves. Our sewage effluent addition experiments resulted in growth rates similar to those observed in bloom situations for both *H. musciformis* and *Ulva fasciata*, which were significantly higher with increasing levels of sewage effluent, whereas no significant difference was found between treatment for *Acanthophora spicifera* and *Dictyota acutiloba*. Therefore, in terms of growth, *H. musciformis* and *U. fasciata* similarly respond to excess nutrients more positively and faster than *A. spicifera* and *D. acutiloba*. Additional results from the sewage effluent addition experiments were that (1) *U. fasciata* requires fewer nutrients to increase photosynthetic performance ($RETR_{MAX}$) than what is required for both *H. musciformis* and *A. spicifera*, (2) *U. fasciata* is more sensitive to decreased nutrient conditions in terms of photosynthetic efficiency (Alpha) than all other species tested, (3) all species, except for *D. acutiloba*, positively respond to excess nutrients in terms of building photosynthetic capacity (E_K) and *U. fasciata* is the most responsive, and (4) the native, non-bloom forming reef plant *D. acutiloba* does not enhance photosynthetic properties in the presence of elevated nutrients, and naturally has higher photosynthetic efficiency than bloom forming algae. Substantial decreases in Nitrogen, Phosphorous, Iron, and Molybdenum were found over a 24hr time period in the *H. musciformis* experiment, which displays the ability of this species to utilize substaintial levels of these nutrients in a short amount of time. In addition, these experiments present the importance of considering more stringent limits on the total allowable daily loads of algal growth promoting macro and micro nutrients, such as Manganese.

References

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Gartner A, Lavery P, Smit AJ (2002) Use of $\delta^{15}N$ signatures of different functional forms of macroalgae and filter feeders to reveal temporal and spatial patterns in sewage dispersal. Mar Ecol Prog Ser 235: 63-73

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Savage C, Elmgren R (2004) Macroalgal (*Fucus vesiculosus*) $\delta^{15}N$ values trace decrease in sewage influence. Ecol App 14(2): 517-526

Umezawa Y, Miyahima T, Yamamuro M, Kayanne H, Koike I (2002) Fine scale mapping of land derived nitrogen in coral reefs by $\delta^{15}N$ in macroalgae. Limnol Oceanogr 47: 1405-1416

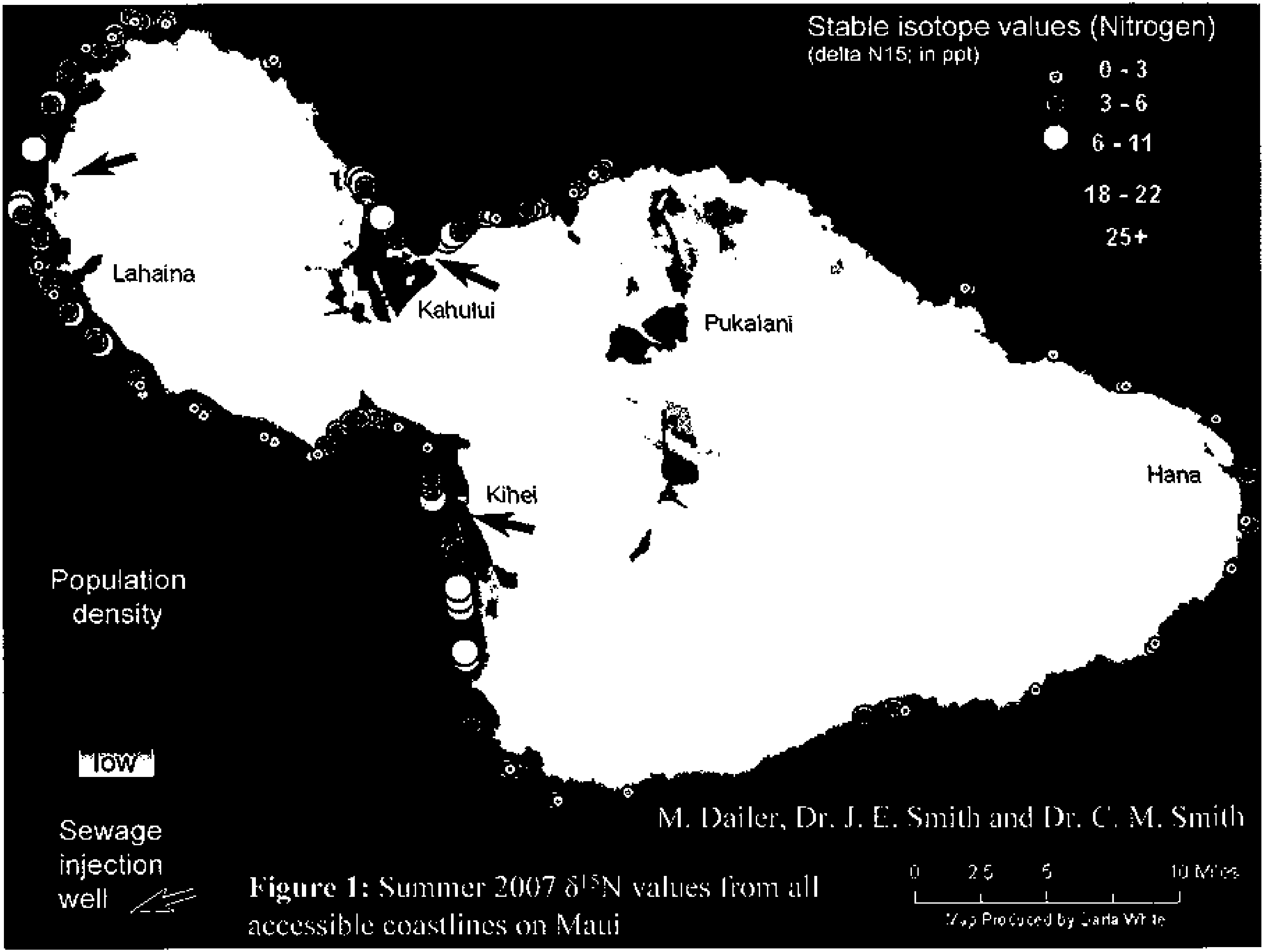


Figure 1: Summer 2007 $\delta^{15}N$ values from all accessible coastlines on Maui

Figure 2: Visual response of *Hypnea musciformis* to the presence or absence of Nitrogen on Day 7 of a reagent grade nutrient enrichment experiment

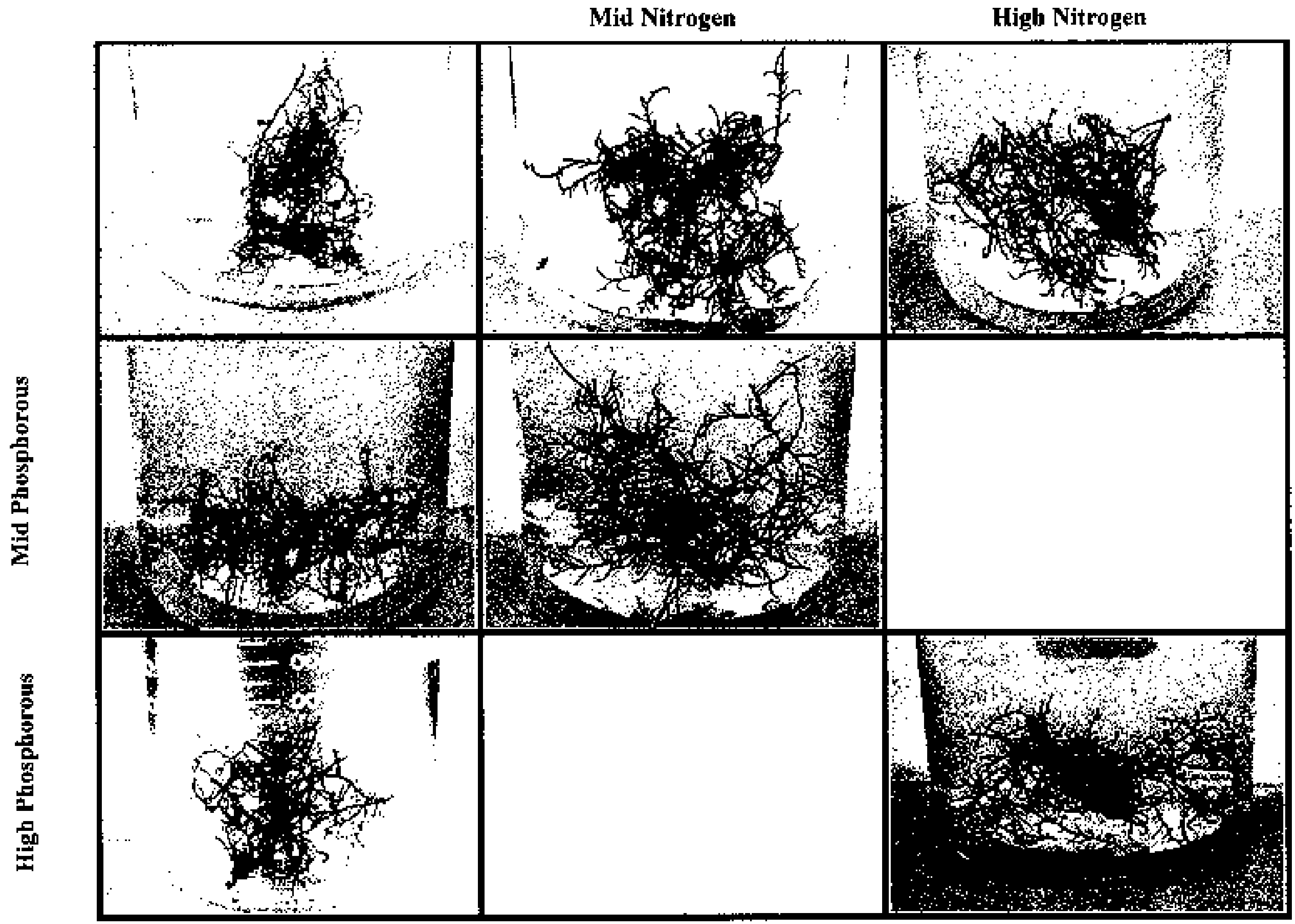


Figure 3: Sequential photographs of individual samples of *Hypnea musciformis* in response to additions of sewage effluent over a period of 9 days

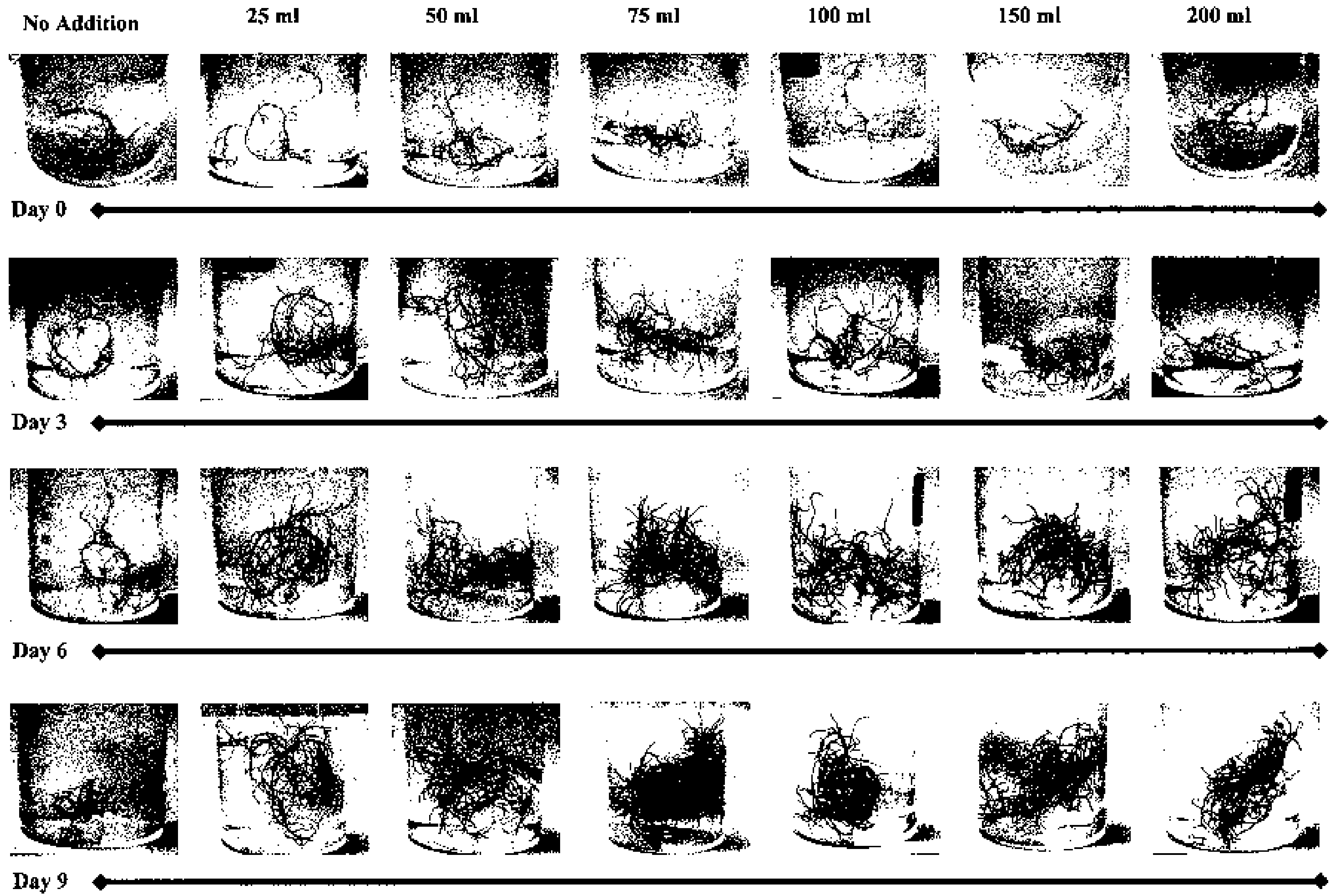


Figure 4: Sequential photographs of individual samples of *Ulva fasciata* in response to additions of sewage effluent over a period of 9 days

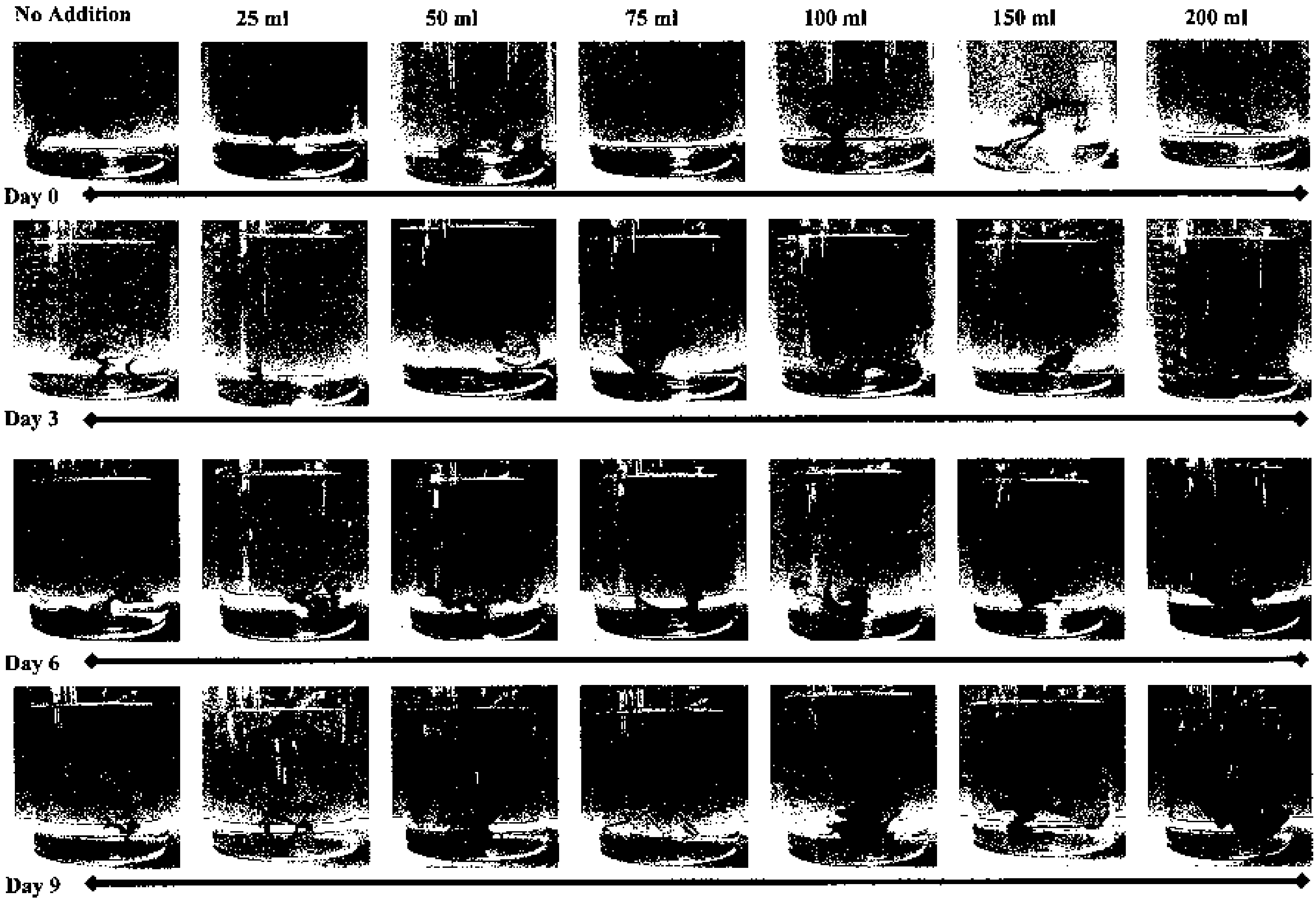


Figure 5: Sequential photographs of individual samples of *Acanthophora spicifera* in response to additions of sewage effluent over 9 days

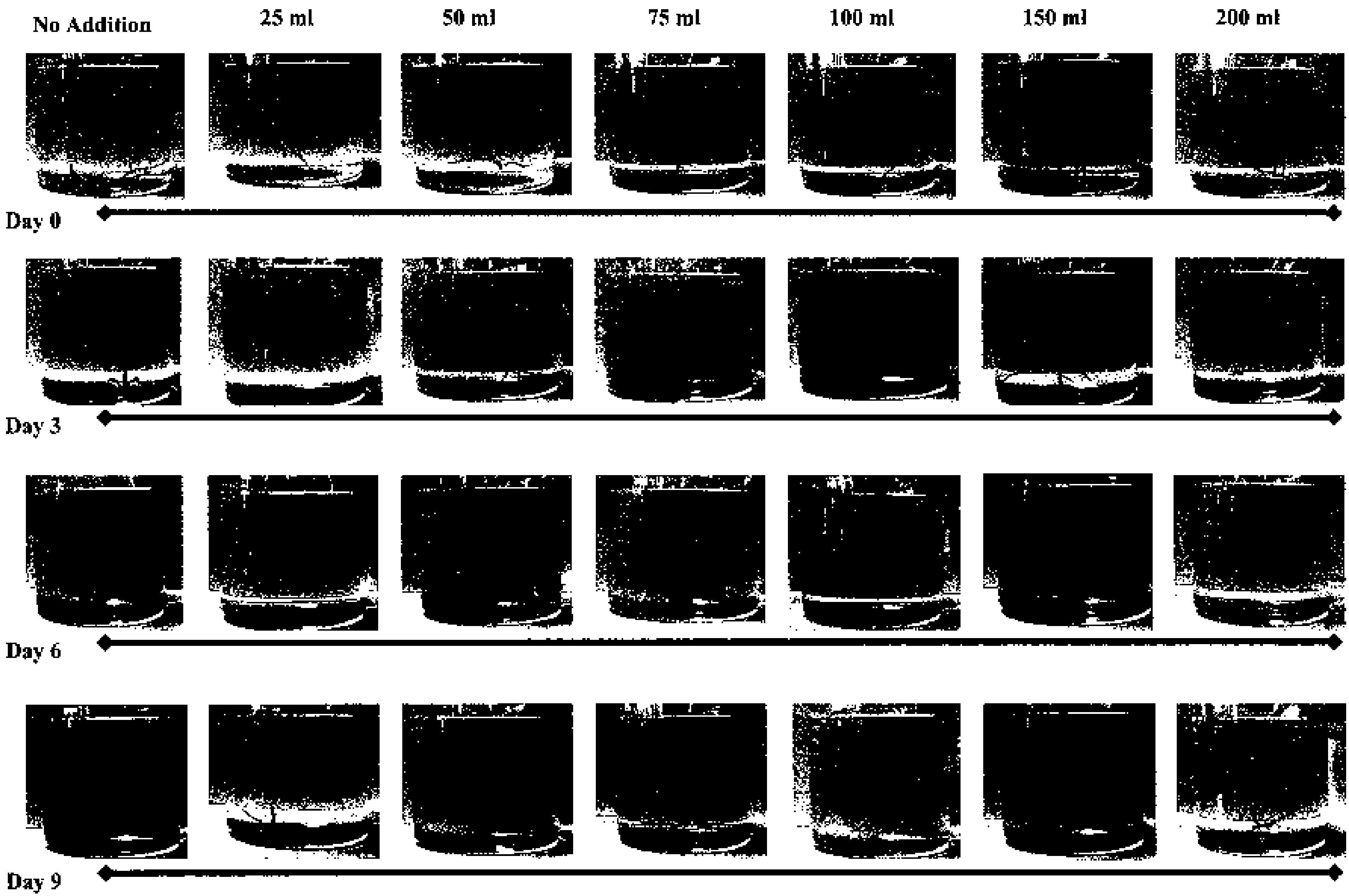


Figure 6: Sequential photographs of individual samples of *Dictyota acutiloba* in response to additions of sewage effluent over a period of 9 days

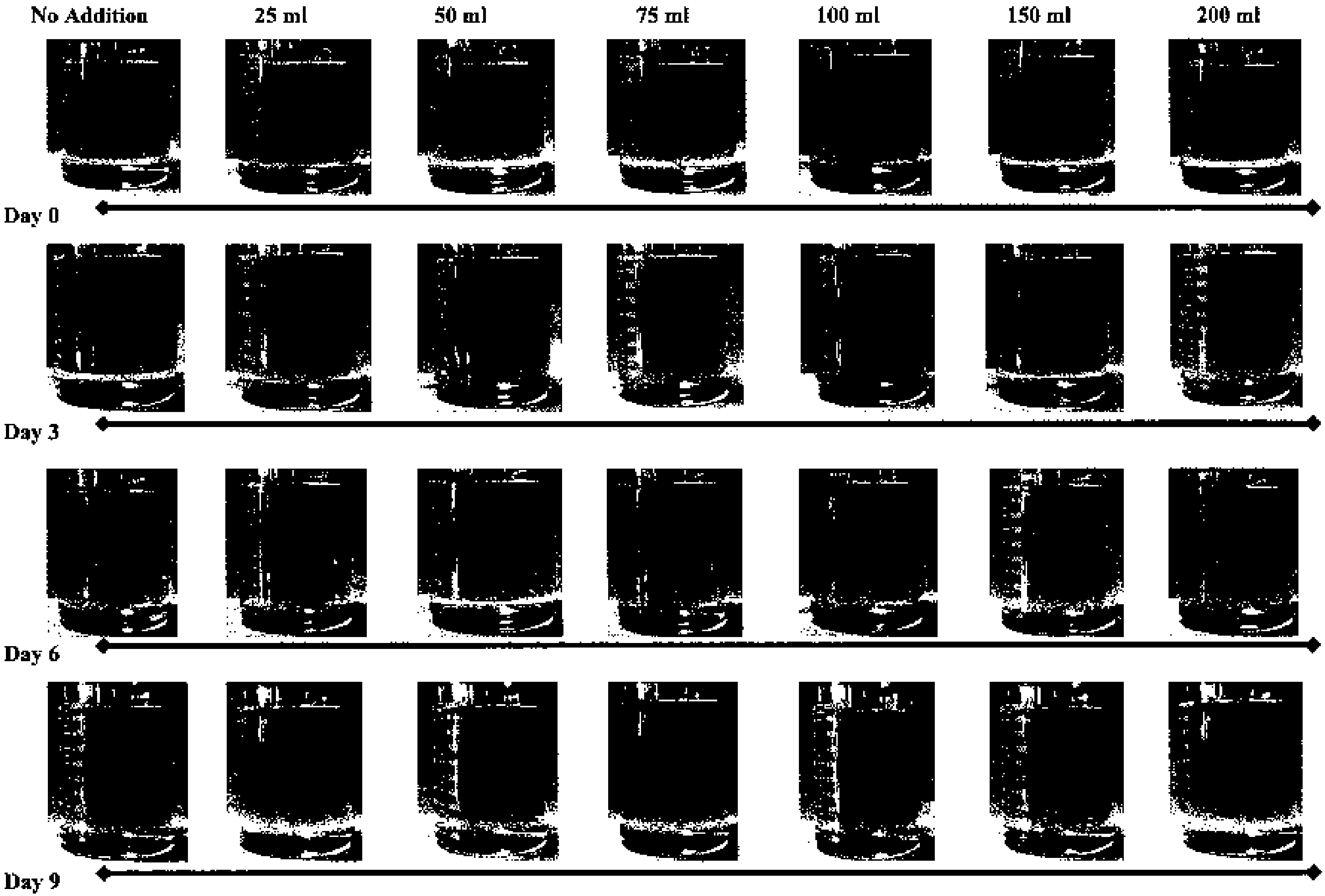


Figure 7A: Relative Growth Rates (RGR) of *Hypnea musciformis*, *Ulva fasciata*, *Acanthophora spicifera*, and *Dictyota acutiloba* in response to additions of sewage effluent for 9 days

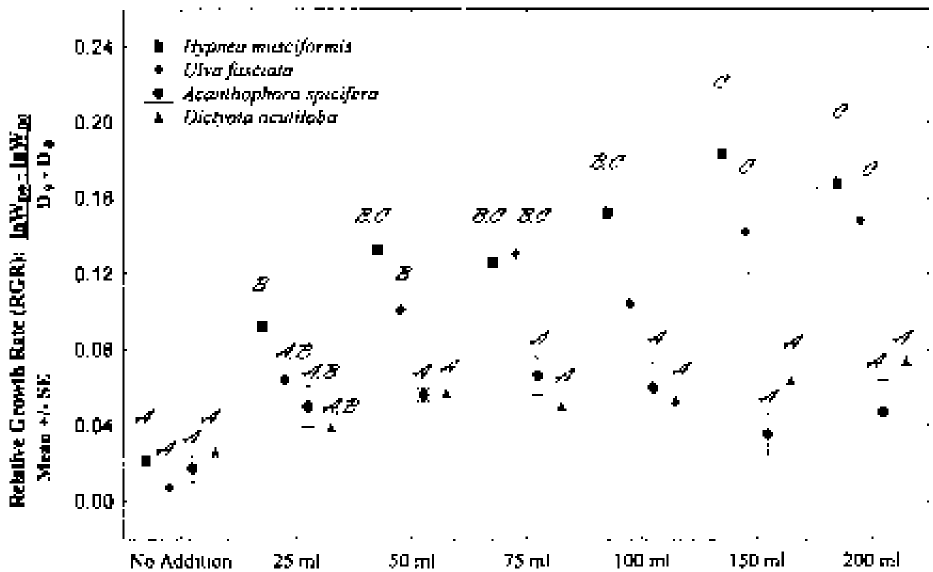


Figure 7C: Day 9 Photosynthetic Saturation Irradiance (E_K) of *H. musciformis*, *U. fasciata*, *A. spicifera*, and *D. acutiloba* in response to additions of sewage effluent

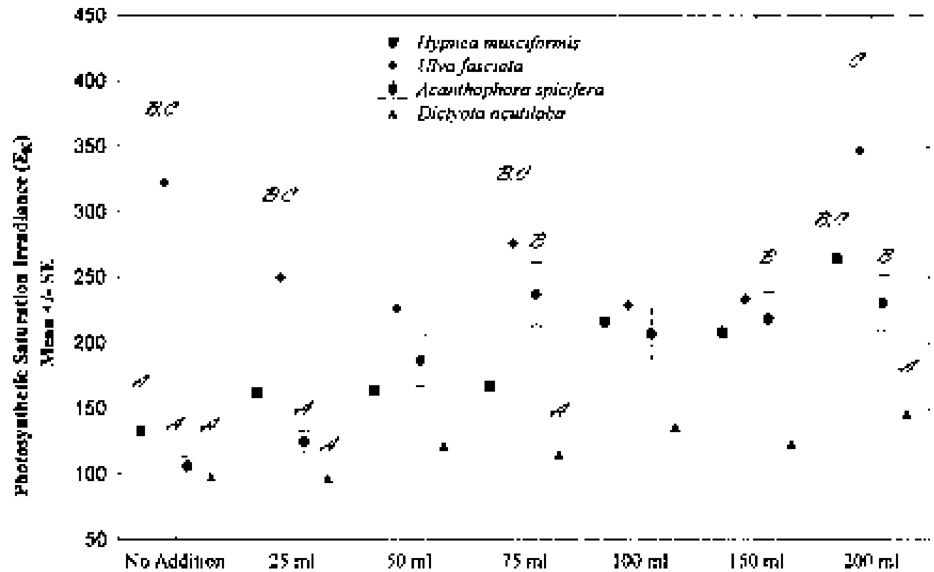


Figure 7B: Day 9 Relative Maximum Electron Transport Rates ($RETR_{MAX}$) of *H. musciformis*, *U. fasciata*, *A. spicifera*, and *D. acutiloba* in response to additions of sewage effluent

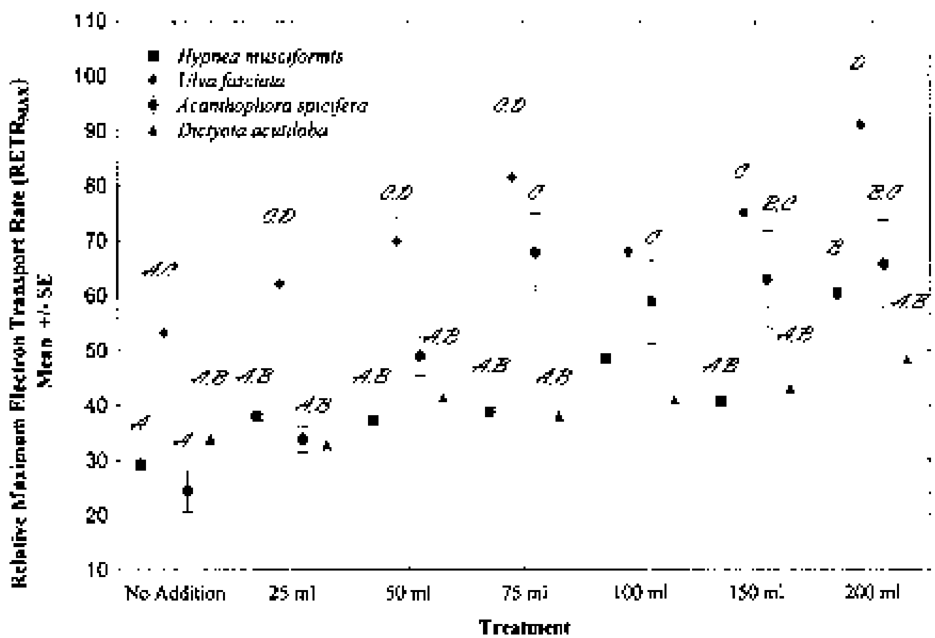
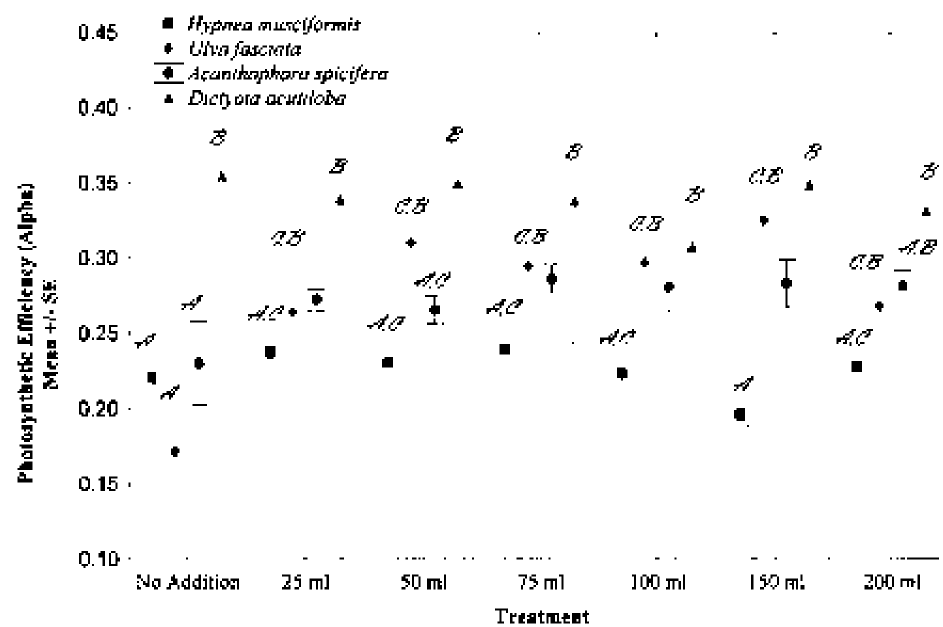


Figure 7D: Day 9 Photosynthetic Efficiency (Alpha) of *H. musciformis*, *U. fasciata*, *A. spicifera*, and *D. acutiloba* in response to additions of sewage effluent



Statistical differences are represented by different letters and comparisons between species are only presented within the same treatment

Figure 8: Water chemistry results from the *Hypnea musciformis* component of the sewage effluent study (A= TOC, B= TP, C= TN, and D= NO_3) Significant differences are represented by different letters. Comparisons between treatments are represented within Day only and comparisons between Days 8 and 9 are represented within treatments only

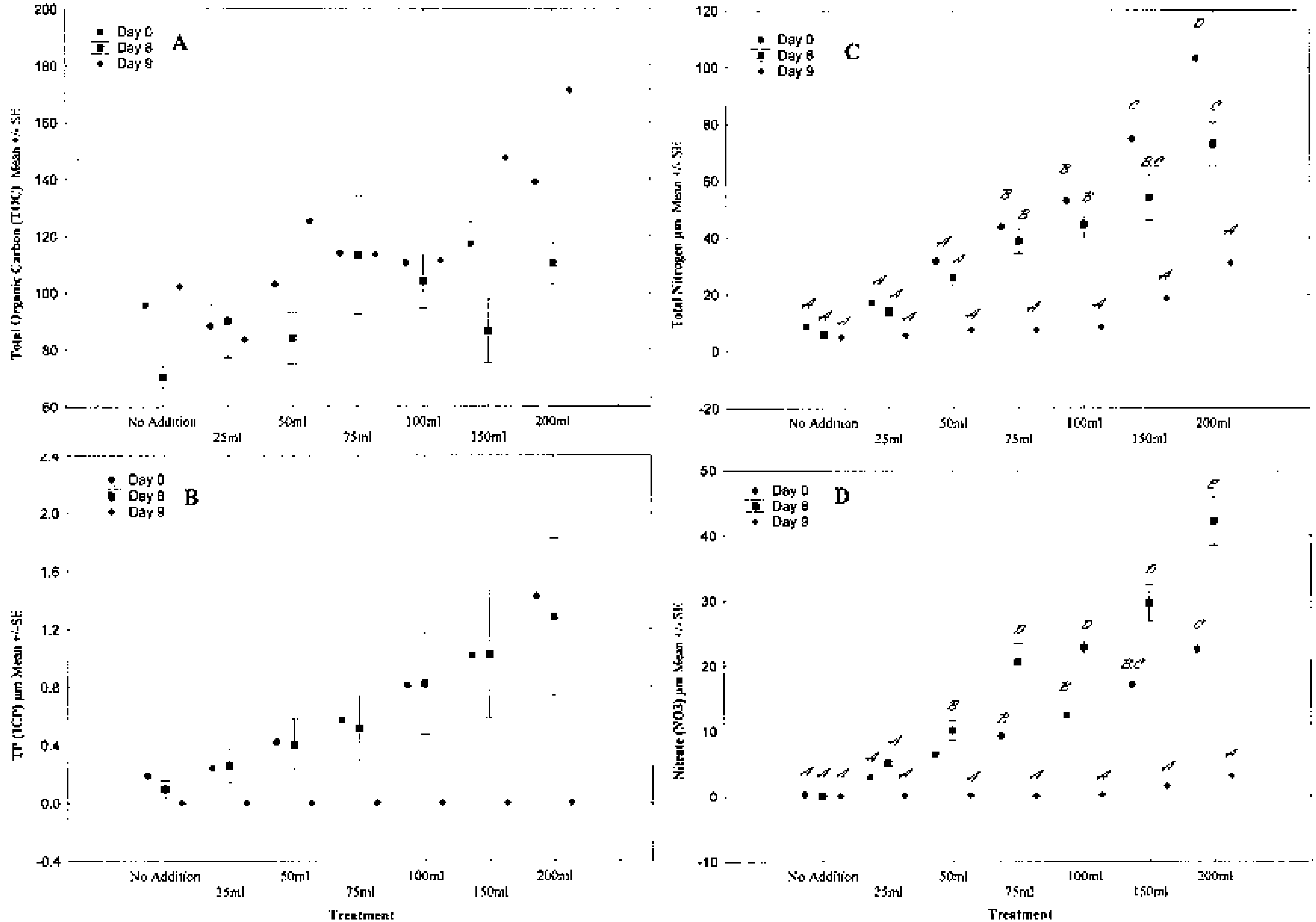


Figure 9: Water chemistry results from the *Hypnea musciformis* component of the sewage effluent study (A= Fe, B= Cu, C= Mn, and D= Mo) Significant differences are represented by different letters. Comparisons between treatments are represented within Day only and comparisons between Days 8 and 9 are represented within treatments only